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IN THE EUROPEAN PATENT OFFICE

In re: European Patent No. : 0 110 385 B1
European Appln. No. : 83 111976.3
Applicant / Proprietor: United States of America, as represented by the Secretary, United States Department of Commerce

DECLARATION OF DR. ENZO PAOLETTI

DR. ENZO PAOLETTI DECLARES AND SAYS THAT:

1. I am advised that Virogenetics Corporation lodged an opposition against the above-referenced patent and application, citing inter alia, the Abstracts and information disseminated publicly at the September 20-23, 1982 Poxvirus-Iridovirus Workshop held at Cold Spring Harbor Laboratory, Cold Spring Harbor, New York (the "September 20-23, 1982 Workshop"). I have been provided with a copy of papers of record in the opposition, which I have reviewed. I am advised and therefore believe that the Applicant/Proprietor has asserted, inter alia, that the Abstracts presented at the September 20-23, 1982 were confidential and, that the first public dissemination of the contents of the article, "Vaccinia virus: A selectable eukaryotic cloning and expression vector," Proc. Natl. Acad. Sci. USA 79:7415-7419 (December 1982) ("the Mackett et al. PNAS article") was by the December 8, 1982 publication of the PNAS article (because distribution of the Abstract ("the Mackett et al. Abstract") and presentation of the same title during the September 20-23, 1982 Workshop was not a public disclosure because of some sort of "confidentiality"). I am further advised and therefore believe that the Applicant/Proprietor has asserted that the contents of the PNAS article and of the Abstract and presentation of the same title during the September 20-23, 1982 Workshop were not disclosed during the American Society for Virology Conference held at Cornell University, Ithaca, N.Y., August 2 to 5, 1982 ("ASV Conference"), despite the August 4, 1982 presentation entitled, "Use of vaccinia virus as a selectable eukaryotic cloning and expression vector" ("the Mackett et al. ASV presentation").

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2. I was in attendance at and a participant in the September 20-23, 1982 Workshop. Note my name on the Participant-Housing List and Author List and, the Abstract at page 55 (and corresponding presentation at the September 20-23, 1982 Workshop), in which I am an author ("the Panicali et al. Abstract"). I have a copy of the Participant-Housing List, Author List and Abstracts from the September 20-23, 1982 Workshop and understand that a copy is also of record as document D4 in the opposition lodged against the above-referenced patent and application. I was also in attendance at the ASV Conference and was an author of a presentation thereat ("the Panicali et al. ASV presentation"). I am also an author of Panicali et al., "Construction of poxvirus as cloning vectors: Insertion of the thymidine kinase gene from herpes simplex virus into the DNA of infectious vaccinia virus," PNAS 79:4927-4931 (August 1982; communicated May 1982; "the Panicali et al. PNAS article").

3. Moreover, a copy of my Curriculum vitae is attached. I was the Director of the laboratory at the Wadsworth Center for Laboratories and Research, New York State Department of Health, Albany, New York that produced the work reported in the Panicali et al. Abstract, the corresponding presentation at the September 20-23, 1982 Workshop, the Panicali et al. ASV presentation and the Panicali et al. PNAS article. That is, the work reported in the Panicali et al. Abstract, the corresponding presentation at the September 20-23, 1982 Workshop, the Panicali et al. ASV presentation and the Panicali et al. PNAS article was done by me or under my direction, supervision and control in the ordinary course of business; and, I was responsible for the content and disclosure of those documents and presentations. Thus, I am the first named inventor in U.S. application Serial No. 334,456, filed December 24, 1981, now U.S. Patent No. 4,769,330, document D9 in the opposition ("the '330 Patent"), from which document D7 in the opposition, EP-A2083 286, claims priority. Accordingly, I am the individual most qualified to address the contents of the Panicali et al. Abstract, the

corresponding presentation at the September 20-23, 1982 Workshop ("the Paoletti laboratory CSH presentation"), the Panicali et al. ASV presentation, the Panicali et al. article, and, the '330 Patent. I am also qualified to speak about events that occurred at the September 20-23, 1982 Workshop and the ASV Conference including the nature of disclosure thereat through presentation of Abstracts and information.

4. Furthermore, with reference to the foregoing and my Curriculum vitae, it is evident that I have had a great deal of experience constructing vaccinia virus recombinants for use as expression vectors, and, was the pioneer in the field, as the Director of the laboratory which produced the work reported in the Panicali et al. Abstract, Paoletti laboratory CSH presentation, the Panicali et al. ASV presentation, the Panicali et al. PNAS article, and the '330 Patent, and, as author or inventor on these documents and presentations, and, as an author or inventor on of numerous other articles or patents in or relating to the field. In the relevant art, at the relevant time, I was skilled; and, am still a skilled artisan in the filed. Thus, I am qualified to render opinions concerning the assertions of the Applicant/Proprietor.

5. I am advised and therefore believe that in support of its assertions, the Applicant/Proprietor relies upon the Agenda for the September 20, 1982 Business and Organizational Meeting of the September 20-23, 1982 Workshop, including, "Should material presented at Pox-Iridovirus Conference be generally referenced in publications?" This reliance is misplaced. The first issue in the Agenda (copy attached) was **not** about whether the "material presented" (Abstracts, presentations and information thereof) was confidential or privileged, but rather that the "material presented" should be referenced as a "personal communication" in publications due to lack of peer review of the material presented. Material presented was nonetheless usable and not confidential or privileged. That is, the first issue of the Agenda only dealt with citation form. I have no recollection

of the issue of confidentiality being addressed or any decision of imposing an obligation of confidentiality or privilege on the "material presented", especially since such an obligation would have been wholly contrary to the concept of the Workshop, namely public dissemination.

6. Moreover, at the time of the September 20-23, 1982 Workshop the Abstracts were assembled (collated) as sequentially numbered pages stapled together. The Abstracts were submitted by presenters prior to the Workshop dates for public dissemination at the Workshop without any promise, express or implied, or other obligation of confidentiality or of any rights reserved by the presenter. The Abstracts were freely distributed, without any obligation of confidentiality and without any reservation of rights by the presenter, at registration for the Workshop; namely, <u>before</u> the first issue of the Agenda was addressed. That is, on the first day of the Workshop (September 20, 1982), before the meeting of that day, the assembled Abstracts were distributed freely at registration for the Workshop, with no obligation of confidentiality or any reservation of rights by the presenter. **Thus, the first issue of the Agenda regarding citation form of the September 20, 1982 meeting is irrelevant. The Abstracts had been publicly disseminated prior thereto without any obligation of confidentiality or privilege and without any reservation of rights by any presenter. Accordingly, contrary to the assertions of the Applicant/Proprietor, as of September 20, 1982, the Abstracts of the September 20-23, 1982 Workshop were publicly disseminated and distributed without any obligation of confidentiality or privilege and without any reservation of rights by the presenter; and the Abstracts were therefore published.**

7. The first issue of the Agenda is also irrelevant in view of the disclosure during the Panicali et al. ASV presentation and the Mackett et al. ASV presentation, as well as the disclosure in the Panicali et al. Abstract and the Panicali et al. PNAS article. The Panicali et al. ASV presentation included contents of the Panicali et al. PNAS

article, the Panicali et al. Abstract and of the Paoletti laboratory CSH presentation. In particular, the Panicali et al. PNAS article, at page 4931 states that: "other (unpublished) data suggest that vaccinia signals may be operative for HSV TK expression [in recombinants vP2, vP4 and vP6]." The Panicali et al. Abstract disclosed that "transcriptional analysis . . . was consistent with endogenous vaccinia promoters." The Paoletti laboratory CSH presentation and the Panicali et al. ASV presentation both detailed the use of recombinant vaccinia virus to express foreign gene products and, that vaccinia promoter was operative in that expression. Indeed, I do not recall any significant differences in content between the Panicali et al. ASV presentation and the Paoletti laboratory CSH presentation. That is, in the Panicali et al. PNAS article, the Panicali et al. ASV presentation, the Panicali et al. Abstract and, the Paoletti laboratory CSH presentation, expression in recombinant vaccinia virus by operation or use of a vaccinia promoter or, expression in recombinant vaccinia virus of exogenous DNA under vaccinia control, was fully disclosed; and, given that the Panicali et al. PNAS article, the Panicali et al. ASV presentation and, the Panicali et al. Abstract were all unquestionably publicly disseminated before the September 20, 1982 meeting of the September 20-23, 1982 Workshop, the first issue of the Agenda of the September 20, 1982 meeting, is irrelevant.

8. Furthermore, the Mackett et al. ASV presentation was very similar in content to the Panicali et al. ASV presentation, and, I do not recall any differences in content in the presentation by Mackett et al. at the September 20-23, 1982 Workshop from the Mackett et al. ASV presentation. Indeed, after observing similar talks by Drs. Panicali and Mackett at the ASV Conference, with Dr. Mackett then speaking after Dr. Panicali, and, in view of the similarities in the Mackett et al. Abstract and the Panicali et al. Abstract, with Dr. Mackett's presentation scheduled in the session before the banquet at the September 20-23, 1982 Workshop whereas Dr. Panicali's presentation was scheduled for the session the

following morning, I tried, albeit unsuccessfully, to have Dr. Panicali's presentation rescheduled for the previous session of the September 20-23, 1982 Workshop (in view of their talks being similar in content, as in the ASV Conference). Thus, it is my distinct recollection that the Mackett et al. ASV presentation included a discussion of "expression" (as in the title of that presentation) and, that that discussion included a disclosure of the use of vaccinia promoters for that expression. Note that the Mackett et al. PNAS article was submitted in the month of the ASV Conference, and, that the Abstracts for the September 20-23, 1982 Workshop were submitted prior to the Workshop dates, such that for "expression" to have been included in the title of the Mackett et al. ASV presentation, it logically follows that Mackett et al. had in hand and disclosed at the ASV Conference their data in the Mackett et al. PNAS article and in the Mackett et al. Abstract of the use of vaccinia promoters for that expression. I likewise note that the Mackett et al. Abstract disclosed the use of a vaccinia promoter for expression of exogenous DNA in a recombinant vaccinia virus and, that that Abstract was also freely disseminated prior to the September 20, 1982 meeting of the September 20-23, 1982 Workshop. Thus, the use of a vaccinia promoter for expression of exogenous DNA in a recombinant vaccinia virus was also disclosed in the Mackett et al. ASV presentation and the Mackett et al. Abstract, before the September 20, 1982 meeting of the September 20-23, 1982 Workshop such that the first issue of the Agenda of the September 20, 1982 meeting is irrelevant.

9. I have read the Declaration of Drs. Panicali and Buller presented by Applicant/Proprietor in the opposition. I note that neither refutes the disclosure by my laboratory at the ASV Conference, or, in the Panicali et al. Abstract and the Paoletti laboratory CSH presentation, of the use of a vaccinia promoter for expression of exogenous DNA in a recombinant vaccinia virus. I also note, upon information and belief, that Dr. Buller, from the time of the September 20-23, 1982 Workshop to a date after his Declaration was with

the National Institutes of Health (NIH), the arm of the assignee of the above-referenced application and patent believed responsible for its licensing. Indeed, I believe that Dr. Buller was in Dr. Moss' laboratory at the NIH and was up for tenure at about the time he gave his Declaration. Further, as shown by Weir et al., PNAS, 1982, 79:1210-1214, at 1210 (D2 in the opposition, which I have read and understood), workers in Dr. Moss' laboratory have freely used material from Poxvirus-Iridovirus Workshops. Indeed in Weir et al., D2 the Abstracts are not cited in the References. Rather, the workers in Dr. Moss' laboratory cited the Abstracts in separate footnotes, as if the material from the Poxvirus-Iridovirus Workshop had been initially incorporated into the article as if it were wholly original to those workers in Dr. Moss' laboratory, with the separate footnotes at page 1210 added after initial submission of the article. In other words, Weir et al., D2 shows that workers in Dr. Moss' laboratory have always considered material presented at Poxvirus-Iridovirus Workshops as free to use, contrary to the Applicant/Proprietor's incredible recreation of history and Dr. Buller's Declaration. Also, attached is a copy of Traktman, Cell, 1990, 62:621-626, publishing details of the International Symposium on Poxvirus and Iridoviruses, held May 15-20, 1990 at the Wintergreen Conference Center as a continuation of the Cold Spring Harbor Workshops, contrary to Dr. Buller's Declaration and the Applicant/Proprietor's assertion "that the confidentiality policy . . . was continued in later Poxvirus-Iridovirus meetings." Note also the acknowledgment to Dr. Buller in the Traktman article, further demonstrating that his Declaration and the Applicant/Proprietor's assertions of confidentiality simply are not true.

10. For various personal and professional reasons, Dr. Panicali did not stay with my laboratory when I moved my laboratory to Virogenetics Corporation, the exclusive licensee of recombinant poxvirus technology of Health Research, Inc.

Dr. Panicali's present company, Therion Biologics Corporation, had unsuccessfully requested a license from Virogenetics Corporation. Dr. Panicali is not qualified to speak about the work disclosed by my laboratory, even if through him, as I was the Director of the laboratory. And, as shown by the attached Notice from the U.S. Federal Register, Vol. 59, page 39572 (Wednesday, August 3, 1994), at about the time Dr. Panicali executed his Declaration, Therion Biologics Corporation was probably involved with the NIH with respect to the use of recombinant vaccinia expressing carcinoembryonic antigen (CEA) or portions thereof. I therefore respectfully submit that Drs. Panicali and Buller were subject to influences and biases which were not disclosed in their Declarations, that their Declarations are factually incorrect and, that in view of those influences and biases and incorrect statements, their Declarations are not credible.

11. I am advised and therefore believe that the Panicali et al. PNAS article may be read alone or with other documents to ascertain the novelty and inventiveness (obviousness) of the subject matter of the claims of the above-referenced patent. I am also advised and therefore believe that document D9, U.S. Patent No. 4,769,330 ("the '330 Patent"), which I have read and understood, may be relied upon for the content of its specification to anticipate the claims in issue in the opposition. I am further advised that the Applicant/Proprietor asserts patentability and attempts to distinguish from Panicali et al. PNAS article as follows (original emphasis omitted):

> Because expression in the Panicali and Paoletti constructs was driven by a fortuitously-proximal vaccinia promoter, there can be no assurance that a vaccinia promoter will be sufficiently proximal and in the proper orientation in relation to an insertion site in *another* insertion site or non-essential region. Moreover, there could be no assurance that other poxviruses would have a transcriptional regulatory sequence sufficiently proximal to the insertion sites in the non-essential regions to drive expression.

> In view of the limitations of the prior art, the problems confronted by inventors Moss, Mackett and Smith was to provide recombinant poxviruses that could reliably and efficiently express foreign genes with proper fidelity. See, for example, pages 4-5 of Application No. 83111976.3 (on which the Patent in suit is based). The inventive solution was to deliberately employ poxvirus transcriptional regulatory sequences to control the expression of the foreign gene in the recombinant poxvirus. To insure that the poxvirus transcriptional regulatory sequence was free to control expression, no non-poxvirus transcriptional regulatory sequences were placed between the foreign gene and the poxvirus transcriptional regulatory sequence. In accordance with a preferred manner of making such a recombinant virus, the poxvirus regulatory sequence was inserted into the poxvirus along with the foreign gene, which insures that the foreign gene and the poxvirus transcriptional regulatory sequence are in the proper orientation relative to one another. By employing this inventive approach, the inventors did not have to rely upon the fortuitous presence of an endogenous promoters near their insertion site.

12. I respectfully submit that the assertions of the Applicant/Proprietor are incorrect. They are, in my view, based upon narrow, misreadings of the prior art, particularly of documents other than the Abstracts and presentations of the September 20-23, 1982 Workshop. The supposed "limitations" and "solution" asserted by Applicant/Proprietor, in my expert opinion, were anticipated by the '330 Patent and were certainly anticipated or suggested by the Panicali et al. PNAS article either alone or in combination with other documents. Such other documents include, Weir et al., D2 (February 1982), and/or Hruby and Ball, "Mapping and Identification of the vaccinia virus Thymidine Kinase Gene," J. Virol., 1982, 43:403-409 (August 1982) ("Hruby 1982") which I have read and understood, and/or Venkatesan et al., Cell 125:805-813, September 1981 (D1, which I have read and understood), together with <u>Molecular Biology of the Gene</u>, p. 714 (3d Ed. by

James Watson, 1976) (a standard textbook), and Pribnow, "Genetic Control Signals in DNA", ch. 7 in Volume 1, "Gene Expression" of Biological Regulation and Development (edited by Robert F. Goldbeyer) ("Pribnow"), 1980, especially Sections 2.1, "The Transcript Unit", and 3.1, "The Promoter," p. 230, 231, alone or with at least one of Rosenberg and Court (1979), "Regulatory sequences involved in the promotion and termination of RNA transcription," Ann. Rev. Genetics 13:319-353 and Breathnach and Chambon (1981), "Organization and expression of eucaryotic split genes coding for proteins," Ann. Rev. Biochem. 50:349-383. For convenient reference, a copy of each of Molecular Biology of the Gene, p. 714, Pribnow, Rosenberg and Court, and Breathnach and Chambon, which I have read and understood, is attached. Also, in this regard, mention is made of Moss et al., "Deletion of a 9,000-Base-Pair Segment of the Vaccinia Virus Genome that Encodes Nonessential Polypeptides," J. Virol., 1981, 40: 387-95 (D21, which I have read and understood), Panicali et al., "Two Major DNA Variants ...", J. Virol., 1981, 37:1000-1010 (which I have read and understood and, a copy of which is attached; "Panicali et al. 1981") and Hruby et al., J. Virol., 1981, 40:456-64 (copy attached and, which I have read and understood; Hruby 1981"), which can be read with the Panicali et al. PNAS article alone or collectively and/or with any of the other documents above-cited.

13. Considering first the Panicali et al. PNAS article and the '330 Patent, the assertions by the Applicant/Proprietor that the Panicali et al. article and the '330 Patent "disclose[] that the inserted HSV TK gene must contain its own herpes promoter in order to obtain transcriptional expression" and that it is somehow novel or nonobvious to "employ poxvirus transcriptional regulatory sequences to control ... expression" are incorrect.

14. The Panicali et al. PNAS article and the '330 Patent disclose the construction of six vaccinia virus HSV TK recombinants and the expression by three of those recombinants. More specifically, the Panicali et al. article

and the '330 Patent disclose VP1 to VP6. VP1, VP3 and VP5 were derived from pDP132 and VP2, VP4 and VP6 were derived from pDP137. The direction of the Bam HSV TK fragment in each of pDP132 and pDP137 were opposite to the other, to thereby obtain expression regardless of whether expression is under the control of HSV DNA acting as a promoter or a vaccinia promoter and, to ascertain logically which of these indeed controlled expression.

15. VP2, VP4 and VP6 all expressed the HSV TK gene, whereas VP1, VP3 and VP5 did not, because of the orientation of the gene in these recombinants. **The Panicali et al. article at page 4931 clearly states: "[T]hat vaccinia signals may be operative for HSV TK expression" in VP2, VP4 and VP6. That is, of the two possibilities, i.e., of whether certain HSV DNA acted as a promoter or whether a vaccinia promoter controlled expression, the Panicali et al. article clearly teaches and suggests that it is the vaccinia signals which are operative.**

16. Moreover, in view of expression by VP2, VP4 and VP6 and not by VP1, VP3 and VP5, the '330 Patent discloses that "**<u>the HSV TK-modified F-fragment is incorporated into the vaccinia variants</u>** in the cell **<u>and is then capable of</u>** replication and **<u>expression under vaccinia control</u>**" (col. 10, lines 7 to 10; emphasis added). This text also appears in D7, EP-A2083 286, which claims priority from U.S. application Serial No. 334,456, filed December 24, 1981 from which the '330 Patent issued. This is clearly a teaching that expression of the foreign gene in the recombinant vaccinia virus (VP2, VP4 and VP6) was by vaccinia (poxvirus) regulatory sequences (control). Also, the '330 Patent in the text at column 2, line 63 to column 3, line 1, teaches, "incorporation, into the mutant [i.e., in the exogenous DNA], of tandem repeats of the gene . . . or of additional genetic elements . . . or . . . the use of a strong promoter"; that is, the '330 Patent clearly teaches that the exogenous DNA can contain several genetic elements, including "a strong promoter". This text also appears in D7. Thus, the '330

Patent clearly teaches a recombinant vaccinia virus wherein there is expression under vaccinia control (promoter) and, wherein the exogenous DNA can contain several genetic elements, including "a strong promoter."

17. There is simply nothing novel or nonobvious in the claims of the above-referenced patent and application (which I have read and understood and, a copy of which are attached), in view of the Panicali et al. article teachings and suggestions and the '330 Patent expressly teaching that expression in vaccinia virus recombinants of the exogenous DNA is under vaccinia control (promoter) and not by virtue of exogenous HSV DNA acting as a promoter, and by expressly teaching that the exogenous DNA can contain several genetic elements, including "a strong promoter" (which means a vaccinia promoter, since expression is taught as under vaccinia control).

18. This conclusion is furthered by considering the state of the art, particularly as shown by the documents cited herein which are of record or attached. In this regard, it is noted that even if the very clear teachings and suggestions and teachings, respectively, of the Panicali et al. PNAS article and of the '330 Patent quoted above, were not in the text of the Panicali et al. PNAS article or the '330 Patent, the probability of that which controlled expression in the constructs VP2, VP4 and VP6 facing the skilled artisan was nothing more than a one of two choice: either exogenous HSV DNA acted as a promoter; or expression of the exogenous DNA was under vaccinia control (promoter); i.e., expression was from only one of two possibilities.

19. I note the disclosure of Panicali et al. 1981 in view of the disclosure of Moss et al. in D21, wherein Moss et al. confirm the observations of Panicali et al. 1981 and, which at 394 states:

> The deletion described here appears to be very similar to the one recently reported by Panicali et al. (17) in a stable "small DNA" variant. Indeed, we suspect that it is identical since their

> serially passaged stock was obtained originally from our laboratory.
>
> The deletion within the vaccinia virus genome had no apparent effect on specific infectivity or virus yield in HeLa cells or plaque size in BSC-1 cells (14). Our preliminary experiments also indicated that variant 6/1 and 6/2 replicated in pig kidney cells (kindly supplied by R. Moyer) in contrast to the host range effects seen with some rabbitpox deletion mutants (16). Therefore, it was of particular interest to determine whether the deletion was in a silent or expressed region of the vaccinia virus genome. Blot hybridization studies of Panicali et al. (17) suggested that the region is an immediate early or early transcriptional unit. Recent translational and transcriptional maps of the left side of the genome reproduced in Fig. 5 indicated that the deleted region encodes a minimum of seven or eight immediate early as well as two minor late polypeptides (4, 25). In this report, the absence of all of these early mRNA's in cells infected with the deletion mutant was established by cell-free translation experiments.

It was certainly not merely fortuitous that a vaccinia promoter was proximal in VP2, VP4 and VP6. Rather, by reviewing orientation of VP1 to VP6 and expression by VP2, VP4 and VP6, it is clear that as stated in the '330 Patent and as taught and suggested in the Panicali et al. PNAS article, expression was under vaccinia control. It was not merely fortuitous that expression was by a proximal vaccinia promoter in the Paoletti et al. PNAS article or the '330 Patent. As shown by Moss et al. 1981 (D21) and Paoletti et al. 1981, the vaccinia genome was known to contain numerous promoters. Note, for instance, that in one region of the vaccinia genome Moss et al. 1981 (D21) confirmed that it was "an immediate early or early transcriptional unit [which] ... encodes a minimum of seven or eight immediate early ... polypeptides." Note again '330 Patent teaching that the exogenous DNA can contain several genetic elements, including "a strong

promoter" (which must be a vaccinia promoter, since the '330 Patent teaches expression under vaccinia control).

20. Note too that as shown in Pribnow, Section 2.1 at p.230 a "transcriptional unit is a stretch of DNA base pairs bounded on one end by a 'start sequence' or *promoter* ... and the other end by a 'stop sequence' or *terminator* "(emphasis in original). Note further that while Applicant/Proprietor admits,

> D1 [Venkatesan et al., Cell 125 805-813 September 1981] discusses the 5' region containing RNA start site of the vaccinia gene encoding a 7.5 Kd polypeptide ... Sequences corresponding to this region were later used to obtain the vaccinia 7.5 K promoter,

it is important to point out that the "5' region containing the RNA start site of the vaccinia gene encoding a 7.5 Kd polypeptide" <u>is</u> "the vaccinia 7.5 K promoter". Thus, the sequence of the 7.5 K promoter <u>was</u> disclosed in D1. No further information other than the information disclosed in D1 was required to "obtain the vaccinia 7.5 K promoter". Accordingly, the Panicali et al. article or the '330 Patent, either alone or in view of D1, anticipated or rendered obvious the claims of the above-referenced patent and application, especially in view of the following detailed discussion of the state of the art.

21. A standard textbook published in 1976 defines a promoter as a "[r]egion on DNA at which RNA polymerase binds and initiates transcription" (p. 714, <u>Molecular Biology of the Gene</u>, 3rd edition, by James Watson). Similarly, in 1980 David Pribnow stated "The basic promoter is only that particular DNA sequence that is recognized directly and used by the RNA polymerase as a start signal for transcription" (Pribnow 1980, section 3.1 "The Promoter", p. 231).

22. The nature of and elements contained within both procaryotic and eucaryotic promoters were well defined before the priority date of the above-captioned patent and application, which I am advised and therefore believe was November 30, 1982. (Rosenberg and Court, 1979; Pribnow 1980;

Breathnach and Chambron, 1981). In particular, it was well established that promoters are commonly located upstream from the ATG initiation of translation sites which begin DNA sequences coding for proteins. In D1, the authors demonstrate extensive familiarity with this knowledge in the state of the art by citing and discussing several publications which reveal elements and characteristics of various procaryotic and eucaryotic promoters (Rosenberg and Court, 1979; Pribnow 1980; Benoist et al., 1980; Flavell et al., 1979; Canaani et al., 1979; Baker et al., 1979; Hashimoto and Green, 1980). The authors of D1 also discuss the occurrence of sequence motifs near the potential AUG initiation of translation sites in the 7.5K mRNA which are similar to sequence motifs found near the initiation of translation codons found in various other virus RNAs (vesicular stomatitis virus RNA, alfalfa mosaic virus RNA 4: Rose 1978; late adenovirus mRNA: Ziff and Evans, 1978; turnip yellow mosaic virus mRNA: Briand et al., 1978).

23. In its title (p. 805) D1 disclosed, "distinctive nucleotide sequence <u>adjacent to</u> multiple initiation ... sites of an early vaccinia virus gene" (i.e., the 7.5 Kd gene; emphasis added).

24. The summary of D1 (p. 805) includes the following:

> A remarkable 88% AT-rich 60 bp DNA sequence was found immediately upstream of the initiation of transcription sites. Although DNA sequences that bear some homology to Pribnow and Hogness boxes are present, additional recognition sequences located further upstream of procaryotic and eucaryotic initiation sites are absent. A possible initiation of translation codon occurs about 50 nucelotides from the 5' end of the message.

25. The preceding quote (in paragraph ~~2~~ 4 EP 2/27/95) clearly describes sequence characteristics of the 7.5 promoter, in comparison to the sequences of canonical procaryotic promoters (which contain a Pribnow box) and eucaryotic promoters (which contain a Hogness box). As the authors in D1 note (p. 810).

> AT-rich sequences previously have been found within the promoter regions of procaryotic and eucaryotic mRNAs (Rosenberg and Court, 1979; Pribnow 1980) . . . Although the AT-rich region near the viral initiation of

EP 2/27/95

transcription site showed some homology to similar regions of procaryotic and eucaryotic genomes, additional homology was not found further upstream.

26. In D1 the authors further note (p. 805), col. 1. lines 7-14) that

> Recently several mRNAs made early after vaccinia virus infection ... and in vitro by virus cores (Venkatesan and Moss, 1981) have been mapped on the vaccinia virus genome. These early mRNAs are not spliced and their cap structures retain the β-^{32}P-label of the initiating nucleotide, indicating the absence of processing at the 5'-end.

27. As the authors of D1 stress (p. 809), referring to the mRNA for the 7.5 polypeptide,

> the β-phosphate of GTP previously was shown to be incorporated into cap structures of this mRNA, providing evidence of the strongest kind that the 5' ends represent true initiation sites (Venkatesan and Moss, 1981)

(i.e., that the 5' end of the mRNA maps to the 7.5 K promoter region).

28. Finally, (at p.811) the authors of D1 conclude

> The multisubunit RNA polymerase of vaccinia virus, like that of procaryotic and eucaryotic organisms, must be capable of interacting with the promoter sequences for a large number of RNAs. Efforts to extend the present studies by sequencing additional genes are in progress.

29. It is thus evident that the DNA sequences disclosed in D1 around the 5' end of the mRNA for the 7.5 polypeptide are in fact and were at the time of publication thereof known to be the promoter sequences for the 7.5 K gene. Thus, the Panicali et al. PNAS article (or the '330 Patent) taken alone or with D1, and alternatively in further combination with Panicali et al. 1981 or Moss et al. 1981 (D21) or with both of Panicali et al. 1981 and Moss et al. 1981 (D21) placed into the skilled artisan's hands:

- recombinant vaccinia virus;
- with the expression of exogenous DNA therein under vaccinia control;

- with the exogenous DNA therein deliberately placed proximinal to vaccinia promoters;

- the 7.5 K promoter; and,

- that the 7.5 K promoter naturally occurs adjacent to the coding sequence for the 7.5 K polypeptide.

30. Accordingly, without inventive effort, one skilled in the art could place desired exogenous DNA proximinal to a vaccinia promoter and, could place a desired promoter such as the 7.5 K promoter adjacent to coding DNA, as such occurs naturally with respect to the 7.5 K promoter and coding sequence for the 7.5 K polypeptide, as shown by D1, when read in the light of the knowledge in the art and the documents D1 itself cites.

31. With respect to Weir et al. D2, since this article discloses that "the vaccinia thymidine kinase gene maps to the 5,000 bp *Hin*dIII J fragment", the "person of skill in the art", who wished to obtain a vaccinia TK promoter would naturally focus his search to sequences within the vaccinia *Hin*dIII J fragment. The "person of skill in the art" would expect that the TK promoter would be located in "upstream sequences of the TK gene". In D2, the authors acknowledge that they were not the only group that had mapped the TK gene to *Hind*III J (D2, p. 1210, col. 2, lines 30-34): "Further evidence that the *Hin*dIII J fragment contains the structural TK gene was obtained in our laboratory and in that of D.E. Hruby and L.A. Ball (personal communication) by cell-free translation of hybridization selected mRNA under conditions suitable for expression of active TK."

32. Prior to November 30, 1982, Drs. Ball and Hruby, in Hruby 1982, reported in detail the location of the vaccinia TK gene. In Hruby 1982, Drs. Ball and Hruby were the first to report that the vaccinia TK gene was transcribed as a 700 nt RNA, and that, contrary to previous expectations, the gene encoded a 19 kilodalton protein. They also reported that "the tk gene lies completely within *Hin*dIII fragment J, and this conclusion is supported by recent analyses of subfragments of J, which show that the gene lies between about

0.5 and 1.2 kilosbases from the L-J boundary". They also disclosed that "the structural gene for VVtk is located in *Hin*dIII fragment J at 42.5 to 45.1 map units". Thus, prior to the above-captioned patent and application, information was available in the public domain which could be used by persons "of skill in the art" to obtain the vaccinia TK promoter, without any inventive efforts. Note also Hruby 1981.

33. Further, the Panicali et al. PNAS article (or the '330 Patent) with Hruby 1982, either alone or in combination with any one or all of D1, Weir et al., D2, Panicali et al. 1981, Moss et al. 1981 (D21) and any other document cited herein of record or attached, placed into the knowledge of the skilled artisan:

- recombinant vaccinia virus;
- with expression of exogenous DNA therein under vaccinia control;
- with exogenous DNA therein deliberately placed proximal to vaccinia promoters;
- the 7.5 K promoter;
- that the 7.5 K promoter naturally occurs adjacent to the coding sequence for the 7.5 K polypeptide; and,
- the mapping and identification of the vaccinia virus thymidine kinase gene.

34. Accordingly, without inventive effort, one skilled in the art: could place desired exogenous DNA proximal to a vaccinia promoter; could place a desired promoter, such as the 7.5 K promoter, adjacent to coding DNA, as such occurs naturally with respect to the 7.5 K promoter and coding sequence for the 7.5 K polypeptide; and, could locate the vaccinia virus TK promoter by looking to sequences upstream from the mapped and identified gene therefor, since promoters for other vaccinia virus genes, such as for the 7.5 K polypeptide, were known to be adjacent to and upstream from the gene.

35. From the literature, it is clear that "deliberately employ[ing] poxvirus transcriptional regulatory

sequences to control expression of the foreign gene" such as by inserting "the poxvirus regulatory sequence ... into the poxvirus along with the foreign gene" was within the ambit of the skilled artisan and, was a simple duplication of that which had already been disclosed, particularly as naturally occurring with respect to the 7.5 K polypeptide gene and the promoter therefor. The exogenous DNA in the Panicali et al. PNAS article and the '330 patent was expressed under vaccinia control and was not "fortuitously-proximal [to a] vaccinia virus promoter" as asserted by the Applicant/Proprietor.

36. While the invention by Dr. Panicali and me, as fully disclosed in the '330 Patent, of a recombinant vaccinia virus (a vaccinia virus synthetically modified to contain exogenous DNA in a nonessential region of the vaccinia virus genome), and particularly of such a virus which has expression under vaccinia control, was not taught or suggested by art pertaining to other vectors, I do submit that the knowledge in the art in 1982 pertaining to the use of promoter elements for expression of heterologous genes using defined, translocated promoter elements can be used in combination with the art discussed above to further evaluate the novelty and nonobviousness of the claims of the above-referenced patent and application and, to also evaluate the credibility of the assertions of the Applicant/Proprietor (since by the '330 Patent, the Panicali et al. PNAS article, the Panicali et al. Abstract, and the Panicali et al. ASV presentation, a recombinant vaccinia virus having expression of exogenous DNA by use of a vaccinia promoter was, by 1982, part of the state of the art). In view of the following review of the state of the art in 1982 of the use of promoter elements for expression of heterologous genes using defined, translocated promoter elements, it is clear that there is nothing inventive in the methodology of the above-referenced patent and application. In particular:

A. Prokaryotic Vectors

The technology of expressing heterologous genes in prokaryotes, specifically in *E. coli*, through the use of

translocated promoter elements was well established prior to 1982. In particular, a number of research groups had demonstrated the expression of eukaryotic genes in *E. coli* through the use of translocated prokaryotic promoter elements. Several examples are described below.

(1) In 1980, Guarente, L., T.M. Roberts, and M. Ptashne published, "A technique for expressing eukaryotic genes in bacteria," Science 209:1428-1430, copy attached, which I have read and understood. In their technique, they insert a small PvuII/PstI "portable promoter fragment" containing the *E. coli lac Z* promoter.

(2) Kupper H., W. Keller, C. Kurz, S. Forss, H. Schaller, R. Franze, K. Strohmaier, O. Marquardt, V.G. Zaslavsky and P.H. Hofschneider (1981), "Cloning of cDNA of major antigen of foot and mouth disease virus and expression in *E. coli*," Nature 289:555-559, copy attached (which I have read and understood), used a small restriction fragment containing the P_L promoter from *E. coli* bacteriophage λ in a plasmid background to direct the expression of the major antigen of foot and mouth disease virus (FMDV).

(3) In October 1982 Watson, R.J., J.H. Weis, J.S. Salstrom and L.W. Enquist (1982), "Herpes simplex virus Type-1 glycoprotein D gene: nucleotide sequence and expression in *Escherichia coli*," Science 218:381-384, copy attached (which I have read and understood), used a "modified λ cro sequence" in a plasmid as a promoter to direct the expression of the HSV-1 glycoprotein D gene in *E. coli*.

(4) Kleid, D.G., D. Yansura, B. Small, D. Dowbenko, D.M. Moore, M.J. Grubman, P.D. McKercher, D.O. Morgan, B.H. Robertson and H.L. Bachrach (1981), "Cloned viral protein vaccine for foot-and-mouth disease: responses in cattle and swine," Science 214: 1125-1129, copy attached (which I have read and understood), reported the use of small, translocated fragments containing the promoter from the *E. coli* try (tryptophan) operon ("tryp-operon") to express FMDV VP_3 in *E. coli*.

(5) Edman, J.C., R.A. Hallewell, P. Valenzuela, H.M. Goodman, and W.J. Rutter. (1981), "Synthesis of hepatitis B surface and core antigens in *E. coli*," Nature 291:503-506, copy attached (which I have read and understood), disclosed the use of small translocated fragments containing the promoter from the *E. coli* tr~~y~~p-operon E.P. 2/21/95 for expression of the hepatitis B core antigen in *E. coli*.

(6) Emtage, J.S., W.C.A. Tacon, G.H. Catlin, B. Jenkins, A.G. Porter and N.H. Carey (1980), "Influenza antigenic determinants are expressed from haemagglutinin genes cloned in *Escherichia coli*," Nature 283:171-174, copy attached (which I have read and understood), disclosed the use of small, translocated fragments containing the promoter from the *E. coli* tr~~y~~p-operon E.P. 2/21/95 to express influenza HA in *E. Coli*.

B. Eukaryotic Vectors

An understanding of the state of the art in 1982 of expressing heterologous eukaryotic genes in eukaryotic cells through the use of translocated eukaryotic promoter elements can be obtained by referring to the book Gluzman, Y. ed. (1982) "*Eukaryotic Viral Vectors*", Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, containing the following, a copy of each of which is attached and, which I have read and understood:

Berkner, K.L. and P.A. Sharp, "Preparation of adenovirus recombinants using plasmids of viral DNA," pp. 193-198.

Fried, M. and E. Ruley, "Use of polyoma virus vector," pp. 67-70.

Gilboa, E., J. Park, M. Kolbe, S. Hwang, R. Kucherlapati, K. Noonan and H. Freeman, "Transduction and expression of nonselectable genes using retrovirus-derived vectors," pp. 145-151.

Gluzman, Y. Preface pp. xI-xII.

Hassell, J.A., C. Mueller, A.M. Mes, M. Featherstone, M. Nanjokas, B. Pomerantz and W. Muller, "The construction of polyoma virus vectors: functions required for gene expression," pp. 71-77.

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Liu, C.C. and A.D. Levinson, "Expression of hepatitis B surface antigen using lytic and nonlytic SV40-based vectors," pp. 55-60.

Southern, P. and P. Berg, "Mammalian cell transformation with SV40 vector, "pp.41-45

The preface to the book, by Yakov Gluzman, of Cold Spring Harbor Laboratory, begins as follows:

> A conference on eukaryotic viral vectors was held at the Banbury Center of the Cold Spring Harbor Laboratory December 3-6, 1981. Approximately 40 DNA and RNA tumor virologists presented and discussed the most recent advances in the use of tumor viruses as cloning vectors.
>
> Progress in this field can be judged by comparing this conference with last year's Banbury meeting on Construction and Use of Mammalian Vectors (December 1980). One year ago, the only viral vectors constructed and used were from SV40 and adenovirus; papilloma and RNA tumor viruses were merely discussed as potential vectors. In contrast, during this meeting, work with a great variety of vectors, including both DNA tumor viruses (SV40, polyoma, papilloma, adenoviruses, herpesvirus) and RNA tumor viruses (MoMLV, MoMSV, HaMSV, ASV, SNV, MMTV), was presented. *Eukaryotic Viral Vectors* summarizes the results of this conference in the form of extended abstracts provided by the speakers. These abstracts are intended to provide the interested reader with an overview of current work in this rapidly progressing field.

Several examples of relevant abstracts published in *Eukaryotic Viral Vectors* are described below.

(1) Two abstracts specifically refer to the movement of small fragments of DNA containing SV40 promoters and other regulatory signals to bacterial plasmids in order to direct the transcription of foreign genes in eukaryotic cells.

> A series of hybrid plasmid vectors has been developed that can be maintained in both bacterial and mammalian cells ... The vectors include sequences from the plasmid pBR322 that permit selection and propagation in *Escherichia coli*. The

> vectors also contain segments of the SV40 genome that constitute a defined eukaryotic transcription unit - promoter, intervening sequence, and polyadenylation site. Foreign DNA sequences (cDNAs or bacterial genes) are inserted adjacent to the SV40 early promoter ... (Southern and Berg, p. 41).

The legend to Figure 1 describes the "structure of the pSV-*neo* hybrid plasmids" which are designed to express the bacterial neomycin resistance gene (the *neo* gene) under the control of the SV40 early promoter. The physical form and location of the promoter are defined:

> The SV40 origin of replication (SV40 ori) EJ 2/27/95 and the SV40 early promoter are present on a small fragment (SV40 map units 0.71-0.65) immediately 5' to the *neo* gene (Southern and Berg, p. 43).

(2) Similarly, in another abstract, Liu and Levinson state:

> Since plasmids containing sequences derived from pBR322 and SV40 are capable of replicating in cells in the presence of SV40 T antigen ... we sought to utilize and extend this system so that foreign gene inserts could be efficiently expressed ... Two fragments of SV40 that span the origin were used. One was 311-bp fragment generated by *EcoRI* digestion of SV40 DNA ... The other was a 348-bp fragment resulting from digestion with both *PvuII* and *HindIII* ... Because the origin region of SV40 encodes the promoters for both the early and late viral transcription units (in opposite orientations), we utilized these fragments in concert with the sequences encoding HBsAg to evaluate their function. Our results indicate that the SV40 promoter, in either the early or late orientation, is capable of efficiently directing the transcription of distal heterologous genes. (Liu and Levinson, pp. 57-58)

Thus, the technology, through the use of restriction endoncleases, of isolating a small restriction fragment containing a promoter from its native location in a particular genome, inserting the fragment in another genetic environment,

and using the promoter contained therein to direct the expression of a heterologous gene, was in the state of the art before November 1982.

(3) Fried and Ruley describe the addition of polyoma virus DNA sequences including the polyoma early promoter into bacterial plasmid pAT153, thus creating a "polyoma virus vector". They describe the use of this hybrid vector to express HSV-TK:

> The herpes 2.4-kb *Eco*RI fragment contains the structural gene for herpes TK but is lacking 5′ control sequences necessary for efficient expression of the gene. This *Eco*RI fragment cloned into the *Eco*RI site of plasmid pAT153 is 20-100 fold inhibited in comparison with the herpes tk 3.6-kb BamHI fragment (cloned in the BamHI site) in converting Ltk-cells to a tk+ phenotype. When the *Eco*RI tk fragment is inserted into the polyoma virus vector at the *Eco*RI site, tk activity is restored (5-25 tk+ colonies/ng of tk gene). Activity is restored only when polyoma control sequences are attached to the 5′ end of the *Eco*RI tk fragment so that polyoma transcription is in the same orientation as the tk gene. (Fried and Ruley, pp. 68-69).

Using their "polyoma virus vector", the authors also demonstrate expression of "the structural gene for human fibroblast interferon ... under polyoma virus control" (p. 69).

(4) Hassell and co-worker describe the "construction of a polyoma virus pBR322 vector suitable for the molecular cloning and characterization of mammalian promoters". As a test of their system, they describe the insertion of SV40 promoters into the hybrid vector and assay for their ability to direct the expression of the polyoma viral oncogene:

> Evidence that foreign promoters can be used to direct expression of the viral oncogene was provided by inserting a fragment of SV40 DNA (the *Hind*III to C fragment) bearing its early and late promoters, upstream of the polyoma viral oncogene ... The resulting recombinant plasmids contain either the SV40 early

> (pSVE1) or late (pSVL1) promoter proximal to the polyoma viral oncogene ... As shown in Table 3, the SV40 early promoter completely restored transforming activity. (Hassell et al., p. 75).

This provides a further example wherein a small restriction fragment containing a promoter can be translocated from its native location in a particular genome and inserted into another genetic environment, where it can function as a promoter to direct the expression of a heterologous gene. In another example, the same authors further report that "preliminary experiments reveal that the major late adenovirus type-2 (Ad2) promoter, when properly juxtaposed before the polyoma viral oncogene, will elicit expression of downstream sequences" (p. 76).

(5) In the process of constructing retrovirus-derived vectors, Gilboa and co-workers describe how they "have identified functionally and isolated physically two viral DNA fragments carrying the *cis*-acting functions required for the expression of the Mo-MLV genes" (Gilboa et al., p. 148) They identified the two Mo-MLV promoters by cloning small fragments of Mo-MLV DNA upstream from HSV-TK coding sequences in a pBR322 background, then testing the resulting plasmids for tk expression by transection of the tk- mouse cells in the presence of HAT selection (which selects for tk activity). In this example also, a translocated promoter element was shown to be functional for the expression of a heterologous gene.

(6) Berkner and Sharp present data on "preparation of adenovirus recombinants using plasmids of viral DNA." They describe the construction of an adenovirus recombinant that contains the positive selection marker dihydrofolate reductase (DHFR) under the control of the adenovirus late promoter. The authors suggest that, with their construct, "subsequent manipulation of the adenoviral late promoter, or even replacement by other putative strong promoters, can be readily evaluated" (Berkner and Sharp, p. 198) Thus, the concepts and techniques of *in vitro* manipulation of promoters and experimental interchange of

different promoters were clearly in the state of the art before November 1982.

(7) A number of studies published prior to November 1982 from the laboratory of Bernard Roizman detail progress in analyzing moveable promoter elements using herpes simplex virus. In some of these studies, the authors inserted a moveable promoter element upstream from HSV-TK coding sequences, then monitored expression of the HSV-TK gene. Using this technique, they demonstrated in 1981 that HSV-TK, which is normally regulated as a β gene, can be expressed as an α gene when placed under the control of a Herpes α promoter derived from the upstream region of the HSV α ICP 4 gene (Post, L.E., S. Mackem and B. Roizman (1981), "Regulation of α genes of herpes simplex virus: expression of chimeric genes produced by fusion of thymidine kinase with α gene promoters," Cell 24:555-565, copy attached, which I have read and understood). In the same publication they demonstrated that the chimeric gene composed of TK coding sequences fused to the α promoter unit could be inserted into the HSV genome, generating a recombinant herpes virus wherein the TK is transcribed under the control of the α promoter. They also demonstrate that the chimeric gene composed of TK coding sequences fused to the α promoter unit could be used to convert LtK- cells to tk+ phenotype. Upon infection of the cell with HSV, the TK coding sequences in the chimeric gene is transcribed under the control of the α promoter, and is regulated as an α gene.

(8) In August 1982, members of the Roizman group reported that a transposed DNA fragment extending 110 nucleotides upstream from the start of RNA transcription for the α ICP 4 gene is sufficient to direct the expression of the TK coding sequences in a chimeric gene, and that a larger DNA fragment, extending beyond 110 nucleotides upstream from the start of RNA transcription, is capable of directing the expression of the chimeric TK gene as an inducible α gene (Mackem, S. and B. Roizman (1982), "Differentiation between α promoter and regulator regions of herpes simplex virus 1: the

functional domains and sequence of a movable α regulator," Proc. Natl. Acad. Sci. USA 79:4917-4921, copy attached, which I have read and understood). In September 1982, the same authors reported that a 325 nucleotide moveable promoter fragment from another HSV α gene, the α gene no. 27, when placed upstream from the HSV-TK coding sequences, was sufficient to specify expression of TK under α regulation (Mackem, S. and B. Roizman (1982), "Regulation of α genes of herpes simplex virus: the α 27 gene promoter-thymidine kinase chimera is positively regulated in converted L cells," J. Virol. 43:1015-1023, copy attached, which I have read and
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un~~g~~erstood).

(9) In March 1982, members of the Roizman group described a plasmid system designed to introduce foreign genes into cells. Plasmid vectors were constructed which contain the HSV-TK gene as a selectable marker, along with coding sequences for the foreign gene placed under the control of a translocated copy of the HSV α 4 promoter. In this technique, Ltk- cells are transfected with the plasmid, then cell lines are selected for conversion to the tk+ phenotype. As an example of this technique, Post et al. demonstrated expression of the chicken ovalbumin gene in transfected cells under the control of the translocated HSV α 4 promoter element (Post, L.E., B. Norrild, T. Simpson, and B. Roizman (1982), "Chicken ovalbumin gene fused to a herpes simplex virus α promoter and linked to a thymidine kinase gene is regulated like a viral gene," Mol. Cell. Biol. 2:233-240, copy attached, which I have read and understood).

C. Vaccinia Virus

In view of the foregoing discussion of the state of the art in 1982 pertaining to the use of promoter elements for expression of heterologous genes using defined, translocated promoter elements, with reference to other Eukaryotic and Prokaryotic vectors, the methodology of the above-referenced patent and application, namely of having the DNA inserted into vaccinia virus containing both a vaccinia promoter for expression and the exogenous gene to be expressed, is neither

novel nor nonobvious; but rather, is the anticipated and obvious extension of the art of the other Eukaryotic and Prokaryotic vectors to vaccinia virus, especially in view of the disclosure in any of the '330 Patent, the Panicali et al. PNAS article, the Panicali et al. Abstract, the Panicali et al. ASV presentation and the Paoletti laboratory CSH presentation (recombinant vaccinia virus with expression of exogenous DNA by use of vaccinia promoter) alone, or, in further view of any of D1 (Venkatesan et al.) (disclosure of "distinctive nucleotide sequence [i.e., the 7.5 Kd promoter] adjacent to multiple initiation ... sites of an early vaccinia virus gene", i.e., the 7.5 Kd gene; emphasis added) and either or both of D2 (Weir et al.) or Hruby 1982 (disclosure of location of vaccinia TK gene from which one skilled in the art could isolate the TK promoter without any inventive effort). Note again the above discussion of each of the '330 Patent, the Panicali et al. Abstract, the Panicali et al. ASV presentation, the Paoletti laboratory CSH presentation, D1, D2 and Hruby 1982. Note particularly that the '330 Patent allows for the DNA insert to include "a strong promoter" (which must be a vaccinia promoter in view of the '330 Patent also teaching expression under vaccinia control) and, that D1, D2 and Hruby 1982 clearly teach enough for the skilled artisan to isolate the vaccinia 7.5 Kd and TK promoters; and, that D1, when read in the light of the state of the art before November 1982 taught that in vaccinia virus promoters are naturally "adjacent to" the gene to be expressed.

37. Thus, the methodology of the above-referenced patent and application is not novel or nonobvious; but rather, is merely a following or a confirmation or an obvious variation of the teachings of any or all of the '330 Patent, the Panicali et al. PNAS article, the Panicali et al. Abstract, the Panicali et al. ASV presentation, and the Paoletti laboratory CSH presentation, alone, or in view of any or all of D1, D2 and Hruby 1982, or, optionally in further view of any or all of the documents concerning other vectors discussed in the preceding paragraph.

38. Therefore, not only do I heartily disagree with the Applicant/Proprietor's recent recreation of the history of the September 20-23, 1982 Workshop, I also strongly disagree with the Applicant/Proprietor's assertions of alleged invention and of the state of the art. The state of the art was far more advanced than asserted by the Applicant/Proprietor and, in view of the state of the art, the Applicant/Proprietor's alleged invention is merely a disclosed or obvious variant of the state of the art and, is by no means any invention.

39. The claims of the above-referenced patent and application and the methodology of the above-referenced patent and application are not novel or nonobvious in view of the Panicali et al. PNAS article or the '330 Patent, either alone or in view of other art such as documents and presentations cited herein and attached or which are already of record. Thus, the above-referenced patent and applications should not have been granted and should be revoked, in its entirety.

I further declare that all statements made herein of my own knowledge are true and that all statements made herein on information and belief are believed to be true.

Date: February 27, 1995

Enzo Paoletti

Dr. Enzo Paoletti